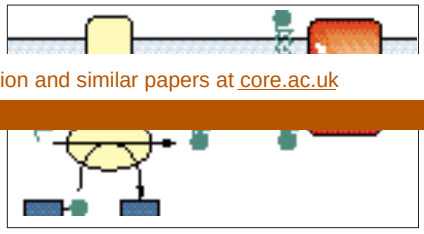
Elsewhere in biology

A selection of interesting papers published last month in *Chemistry & Biology*'s sister journals, *Current Biology* and *Structure* with *Folding & Design*, chosen and summarized by the staff of *Chemistry & Biology*.

Chemistry & Biology 2000, **7**:R123–R126

☐ **Specific proteins are required to translocate phosphatidylcholine bidirectionally across the endoplasmic reticulum.**
Anant K Menon, William E Watkins III and Sigrún Hrafnsdóttir (2000). *Curr. Biol.* **10**, 241–252.

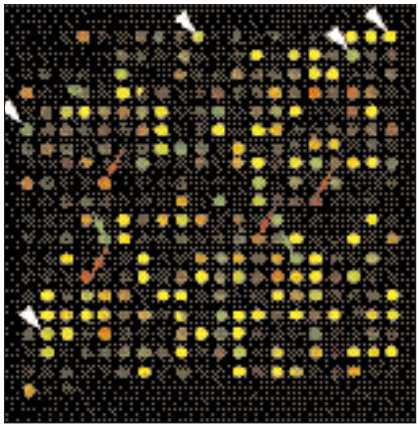
A long-standing problem in understanding the mechanism by which the phospholipid bilayer of biological membranes is assembled concerns how phospholipids flip back and forth



between the two leaflets of the bilayer. This question is important because phospholipid biosynthetic enzymes typically face the cytosol and deposit newly synthesized phospholipids in the cytosolic leaflet of biogenic membranes such as the endoplasmic reticulum (ER). These lipids must be transported across the bilayer to populate the exoplasmic leaflet for membrane growth. Transport does not occur spontaneously and it is presumed that specific membrane proteins — flippases — are responsible for phospholipid flip-flop. No biogenic membrane flippases have been identified. To test the hypothesis that specific proteins facilitate phospholipid flip-flop in the ER, the authors reconstituted transport-active proteoliposomes from detergent-solubilized ER vesicles under conditions in which protein-free liposomes containing ER lipids were inactive. Transport measurements and chromatographic analyses showed that specific proteins are required to translocate a phosphatidylcholine analogue across the ER membrane. These proteins are likely to be the flippases, which are required to translocate natural phosphatidylcholine and other phospholipids across the ER membrane.
21 February 2000, Research Paper, *Current Biology*.

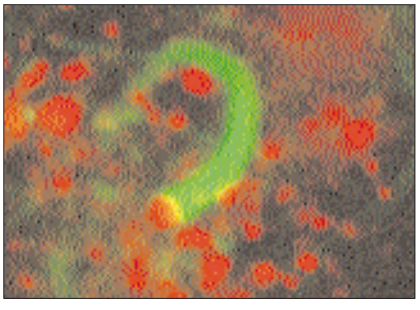
☐ **Microarray analysis of the transcriptional network controlled by the photoreceptor homeobox gene *Crx*.**
FJ Livesey, T Furukawa, MA Steffen, GM Church and CL Cepko (2000). *Curr. Biol.* **10**, 301–310.

Terminal differentiation of many cell types is controlled and maintained by tissue- or cell-specific transcription factors. Little is known, however, about the transcriptional networks controlled by such factors and how they regulate pivotal role in the terminal differentiation of vertebrate photoreceptors. Mutations in the human *CRX* gene result in either congenital blindness or photoreceptor degeneration and targeted mutation of the mouse *Crx* results in failure of development of the light-detecting outer segment of photoreceptors. The authors have characterized the transcriptional network controlled by Crx by microarray analysis of gene expression in developing retinal tissue from $Crx^{+/+}$ and $Crx^{-/-}$ mice. These data were combined with analyses of gene expression in developing and adult retina, as well as adult brain. The most abundant elements of this network are ten photoreceptor-specific or -enriched genes, including six phototransduction genes. The study shows that cDNA microarrays can be used to define the transcriptional networks controlled by transcription factors in vertebrate tissue *in vivo*.
6 March 2000, Research Paper, *Current Biology*.



☐ **Phosphatidylinositol 4,5-bisphosphate induces actin-based movement of raft-enriched vesicles through WASP–Arp2/3.**
AL Rozelle, LM Machesky, M Yamamoto, MHE Driessens, RH Insall, MG Roth, K Luby-Phelps, G Marriott, A Hall and HL Yin (2000). *Curr. Biol.* **10**, 311–320.

Phosphatidylinositol 4,5-bisphosphate vesicle trafficking. It stimulates *de novo* actin polymerization by activating the pathway involving the Wiskott–Aldrich

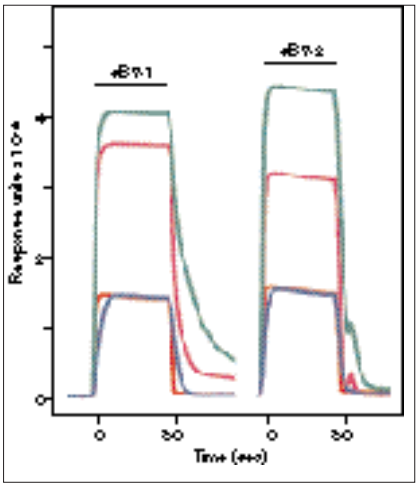


syndrome protein (WASP) and the actin-related protein complex Arp2/3. Actin polymerizes from cholesterol–sphingolipid-rich membrane microdomains called 'rafts', in a manner dependent on tyrosine phosphorylation. Although actin has been implicated in vesicle trafficking, and rafts are sites of active phosphoinositide and tyrosine kinase signaling that mediate apically directed vesicle trafficking, it is not

known whether phosphoinositide regulation of actin dynamics occurs in rafts, or if it is linked to vesicle movements. The authors show that sphingolipid–cholesterol rafts are preferred platforms for membrane-linked actin polymerization. This is mediated by *in situ* PIP_2 synthesis and tyrosine kinase signaling through the WASP–Arp2/3 pathway. Actin comets may provide a novel mechanism for raft-dependent vesicle transport and apical membrane trafficking.
7 March 2000, Research Paper, *Current Biology*.

☐ **LICOS, a primordial costimulatory ligand?**
D Brodie, AV Collins, A Iaboni, JA Fennelly, LM Sparks, X-N Xu, PA van der Merwe and SJ Davis (2000). *Curr. Biol.* **10**, 333–336.

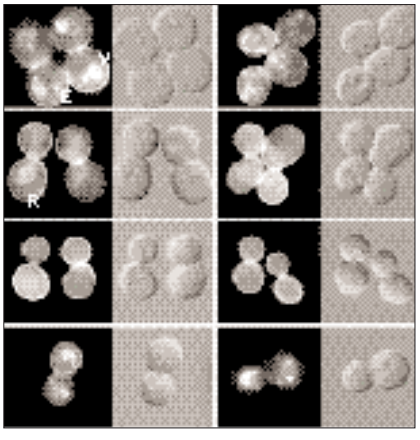
In mammals, the classical B7 molecules expressed on antigen-presenting cells, B7-1 (CD80) and B7-2 (CD86), bind the structurally related glycoproteins CD28 and CTLA-4 (CD152), generating costimulatory signals that regulate the activation state of T cells. A recently identified human CD28-like protein, ICOS, also induces costimulatory signals in T cells when cross-linked with antibodies, but it is unclear whether ICOS is part of a B7-mediated regulatory



pathway of previously unsuspected complexity, or whether it functions independently and in parallel. The authors report that, rather than binding B7-1 or B7-2, ICOS binds a new B7-related molecule of previously unknown function that they call LICOS. The results define the components of a distinct and novel costimulatory pathway and also raise the possibility that LICOS, rather than B7-1 or B7-2, is the contemporary homologue of a primordial vertebrate costimulatory ligand.
10 March 2000, Brief Communication, *Current Biology*.

☐ **G-protein-coupled receptors function as oligomers *in vivo*.**
Mark C Overton and Kendall J Blumer (2000). *Curr. Biol.* **10**, 341–344.

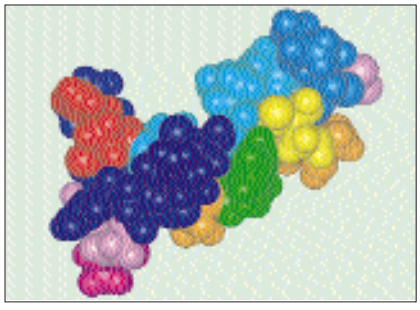
Hormones, sensory stimuli, neurotransmitters and chemokines signal by activating G-protein-coupled receptors (GPCRs). Although GPCRs are thought to function as monomers, they can form dimers, and coexpression of two nonfunctional or related GPCRs can result in rescue of activity or modification of function. Dimerization of peptides corresponding to the third cytoplasmic loops of GPCRs increases their potency as activators of G proteins *in vitro*, and peptide inhibitors of dimerization diminish β_2-adrenergic receptor signaling. It is not known whether GPCRs exist as monomers or oligomers in intact cells and membranes, whether agonist binding regulates monomer–oligomer equilibrium, or whether oligomerization governs GPCR function. The authors report that the α-factor receptor, a GPCR that is the product of the *STE2* gene in the yeast *Saccharomyces cerevisiae*,



is oligomeric in intact cells and membranes. They suggest that oligomerization is likely to govern GPCR signaling and regulation.
10 March 2000, Brief Communication, *Current Biology*.

☐ **NMR structure of an anti-gp120 antibody complex with a V3 peptide reveals a surface important for co-receptor binding.**
Vitali Tugarinov, Anat Zvi, Rina Levy, Yehezkiel Hayek, Shuzo Matsushita and Jacob Anglister (2000). *Structure* **8**, 385–396.

The envelope glycoprotein of human immunodeficiency virus type-1 (HIV-1), which consists of an external domain, gp120, and a transmembrane domain, gp41, is the most important target for the humoral immune response that is elicited by an anti-HIV vaccine. The protein 0.5β is a potent strain-specific HIV-1 neutralizing antibody raised against the entire gp120 of the $HIV\text{-}1_{IIIB}$ strain. The epitope recognized by 0.5β is located within the third hypervariable region (V3) of gp120. Recently, several



HIV-1 V3 residues involved in co-receptor utilization and selection were identified. The nuclear magnetic resonance (NMR) results presented in this paper suggest that the surface of the V3 peptide in contact with the antibody is likely to correspond to a solvent-exposed region in the native gp120 molecule. The conformation of the V3IIIB peptide bound to 0.5 consists of two antiparallel strands linked by a QRGPGR loop (single-letter amino acid code). The structure of the binding site of the anti-gp120 antibody in complex with the V3 peptide can be used to

analyze the structural and functional implications of sequence variations on co-receptor usage and antibody specificity. This structure might help to construct a cocktail of V3 peptides that will induce a broadly neutralizing immune response. In the absence of the three-dimensional structure of the chemokine receptor involved in HIV-1 infection, the structure of V3 might be useful to design anti-HIV-1 drugs that target this receptor.
24 March 2000 Research Paper, *Structure*.

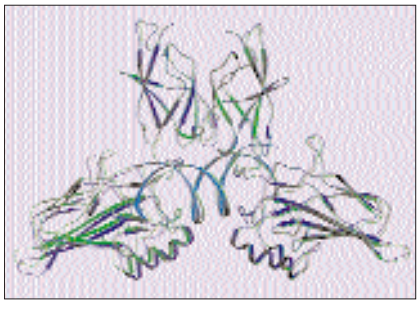
☐ **Solution structure of PCP, a prototype for the peptidyl carrier domains of modular peptide synthetases.**
Thomas Weber, Roland Baumgartner, Christian Renner, Mohamed A Marahiel and Tad A Holak (2000). *Structure* **8**, 407–418.

Nonribosomal peptide synthetases (NRPSs) are large modular enzymes responsible for the synthesis of a variety of microbial bioactive peptides. They consist of modules and each one recognises and incorporates one specific amino acid into the peptide product. A module comprises several domains, which carry out the individual reaction steps. After activation by the adenylation domain, the amino acid

substrate is covalently tethered to a 4′-phosphopantetheinyl cofactor of a peptidyl carrier domain (PCP) that passes the substrate to the reaction centres of the consecutive domains. The solution structure of PCP, a distinct peptidyl carrier protein derived from the equivalent domain of an NRPS, was solved using NMR techniques. PCP is a distorted four-helix bundle with an extended loop between the first two helices. Its overall fold resembles the topology of acyl carrier proteins (ACPs) from *Escherichia coli* fatty acid synthase and actinorhodin polyketide synthase from *Streptomyces coelicolor*, but the surface polarity and the length and relative alignment of the helices are different. The conserved serine — the cofactor-binding site — is situated within a stretch of seven flexible residues. The structure of PCP reflects its character as a protein domain. The flexibility of the post-translationally modified site might have implications for interactions with the cooperating proteins or NRPS domains.
29 March 2000, Research Paper, *Structure*.

☐ **NF-κB p65 (RelA) homodimer uses distinct mechanisms to recognize DNA targets.**
Yong-Qing Chen, Lei Lei Sengchanthalangsy, Arthur Hackett and Gourisankar Ghosh (2000). *Structure* **8**, 419–428.

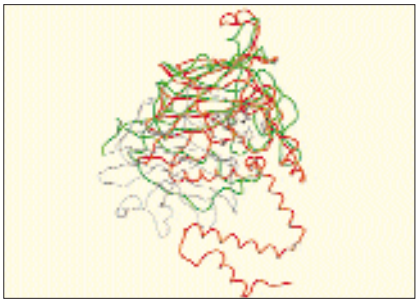
The NF-κB family of dimeric transcription factors regulates the expression of several genes by binding to a variety of related DNA sequences. One of these dimers, p65(RelA), regulates a subclass of these targets. p65 binds to the 5′-GGAA T TTTC-3′ sequence asymmetrically. In that complex, one subunit base specifically interacts with the preferred 5′ half site and the other subunit binds nonspecifically to the 3′ half site. The crystal structures of two new p65–DNA complexes are described in this paper. The structures reveal that p65 exhibits the unique capability to specifically bind DNA targets of variable lengths



from four to ten base pairs. Also, the small protein segment Arg41–Ser42–Ala43 is at least partially responsible for flexibility in DNA-binding modes.
30 March 2000, Research Paper, *Structure*.

☐ **The 1.8 Å crystal structure of catechol 1,2-dioxygenase reveals a novel hydrophobic helical zipper as a subunit linker.**
Matthew W Vetting and Douglas H Ohlendorf (2000). *Structure* **8**, 429–440.

Intradiol dioxygenases catalyze the critical ring-cleavage step in the conversion of catecholate derivatives to citric acid cycle intermediates. Catechol 1,2-dioxygenases (1,2-CTDs) have a rudimentary design structure — a homodimer with one catalytic non-heme ferric ion per monomer, that is $(\alpha Fe^{3+})_2$. This is in contrast to the archetypical intradiol dioxygenase protocatechuate 3,4-dioxygenase (3,4-PCD), which forms more diverse oligomers, such as $(\alpha\beta Fe^{3+})_{2-12}$. The crystal structure of 1,2-CTD from *Acinetobacter sp.* ADP1

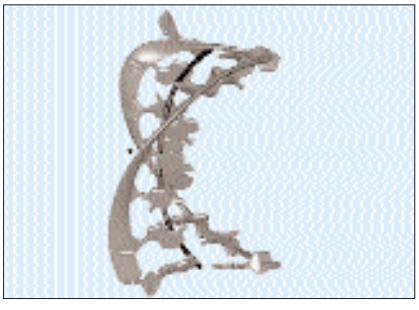


(Ac 1,2-CTD) was solved and the structures of the enzyme complexed with catechol and 4-methylcatechol were also determined. Although the characteristics of the iron ligands are similar, Ac 1,2-CTD differs from 3,4-PCDs in that only one subunit is used to fashion each active-site cavity. In addition, a novel 'helical zipper', consisting of five amino-terminal helices from each subunit, forms the molecular dimer axis. Two phospholipids were unexpectedly found to bind within a hydrophobic tunnel along this axis. The helical zipper domain of Ac 1,2-CTD has no equivalent in other proteins of

known structure. Sequence analysis suggests the domain is a common motif in all members of the 1,2-CTD family. The structures presented in this paper are the first of a new family of intradiol dioxygenases.
30 March 2000 Research Paper, *Structure*.

☐ **A high-resolution structure of a DNA–chromomycin–Co(II) complex determined from pseudocontact shifts in nuclear magnetic resonance.**
Miriam Gochin (2000). *Structure* **8**, 441–452.

The drug chromomycin-A_3 binds to the minor groove of DNA and requires a divalent metal ion for complex formation. ^{1}H, ^{31}P and ^{13}C pseudocontact shifts occurring in the presence of a tightly bound divalent cobalt ion in the complex between $d(TTGGCCAA)_2$ and chromomycin-A_3 have been used to determine the structure of the complex. The accuracy of the structure was verified by validation with nuclear Overhauser enhancements (NOEs) and J-coupling constants not used in the structure calculation. The structure was compared with a structure obtained in an earlier study using NOEs to assess the accuracy of NOEs in giving global structural information for a DNA complex. Although some basic features of the



structures agreed, they differed substantially in the fine structural details and in the DNA axis curvature generated by the drug. The distortion of base-pair planarity that was observed in the NOE structure was not seen in the structure determined in this paper. Differences in drug orientation and hydrogen bonding also occurred. The curvature and elongation of the DNA that was obtained previously was not found to occur. The use of pseudocontact shifts has enabled the authors to obtain a high-precision global structure of the chromomycin–DNA complex, which provides an accurate template on which to consider targeting minor groove binding drugs. The effect of such binding is not propagated far along the helix but is restricted to a local kink in the axis that reverts to its original direction within four base pairs.
30 March 2000, Research Paper, *Structure*.